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PHOSPHOLIPID REQUIREMENTS OF Ca^{++}-STIMULATED, Mg^{++}-DEPENDENT ATP HYDROLYSIS IN RAT BRAIN SYNAPTIC MEMBRANES

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The phospholipid requirement for Ca^{++}-stimulated, Mg^{++}-dependent (ATP) hydrolysis (Ca^{++}/Mg^{++}-ATPase) and Mg^{++}-stimulated ATP hydrolysis (Mg^{++}-ATPase) in rat brain synaptosomal membranes was studied employing partial delipidation of the membranes with phospholipase A_2 (hog pancreas), phospholipase C (Bacillus cereus), and phospholipase D (cabbage). Treatment with phospholipase A_2 caused an increase in the activities of Ca^{++}/Mg^{++}-ATPase and Mg^{++}-ATPase; whereas, with phospholipase C treatment, both the enzyme activities were inhibited. Phospholipase D treatment had no effect on Ca^{++}/Mg^{++}-ATPase, but Mg^{++}-ATPase activity was inhibited. Inhibition of Mg^{++}-ATPase activity after phospholipase C treatment was relieved with the addition of phosphatidylinositol-4,5-biphosphate (PIP_2) and to a lesser extent with phosphatidylinositol-4-phosphate (PIP) and phosphatidylcholine (PC). Phosphatidylserine (PS), phosphatidic acid (PA), PIP, and (continued on reverse)

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19. ABSTRACT (continued)

PIP_2 brought about the reactivation of Ca^{++}/Mg^{++}-ATPase activity. The Michaelis constants (K_{ms}) for Ca^{++} (0.47 μM) and Mg^{++} (60 μM) of the enzyme was unaffected after treatment with the phospholipases.

PREFACE

The work described in this report was authorized under Contract No. DAAK11-84-K-0003. This work was started in June 1984 and completed in December 1985.

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals" as promulgated by the committee on Revision of the Guide for Laboratory Animals Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council.

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PHOSPHOLIPID REQUIREMENTS OF Ca^{++}-STIMULATED, Mg^{++}-DEPENDENT ATP HYDROLYSIS IN RAT BRAIN SYNAPTIC MEMBRANES

1. INTRODUCTION

Ca^{++} is required for various important processes in the nerve cell, and its concentration at the micromolar level is maintained by:

- Sequestration into mitochondria
- Na^{+}-Ca^{++} exchange
- Pumping of Ca^{++} by Ca^{++}/Mg^{++}-ATPase against concentration gradient

The maintenance of cytosolic Ca^{++} levels within the nerve terminal is important for transmitter release and other events in synaptic transmission. Therefore, the enzymes necessary to maintain Ca^{++} levels in the cytosol act as major steps for autoreceptors, transmitters, and drugs. The lipid dependence of such enzymes becomes important in understanding the molecular mechanisms for receptor/effector systems and in isolating and reconstituting such systems.

High affinity Ca^{++}-stimulated, Mg^{++}-dependent ATPase from rat brain synaptosomes[1,2] is a calmodulin-dependent integral membrane-bound protein.[3-5] Most of the membrane-bound enzymes are lipid-dependent, and phospholipases have been used as popular tools to study the lipid requirement of these proteins. Ca^{++} + Mg^{++}-ATPase from erythrocytes[6-10] and sarcoplasmic membrane[11-13] are phospholipid dependent. Choquette et al.[10] also report stimulation of rat brain synaptosomal Ca^{++}/Mg^{++}-ATPase by polyphosphoinositides. To understand the phospholipid dependency of the two components of this Ca^{++}-pumping enzyme system, we partially delipidated the synaptosomal membranes with various phospholipases. Different effects of such treatments and of phospholipids on Ca^{++}/Mg^{++}-ATPase and Mg^{++}-ATPase are reported.

2. METHODS

2.1 Materials.

Phospholipase A_2 (hog pancreas), phospholipase C (*Bacillus cereus*), and phospholipase D (cabbage) were from Boehringer Mannheim Biochemicals. L-α-Phosphatidic acid (PA), phosphatidylcholine (Type IIIE from egg yolk), L-α-phosphatidylethanolamine [PE (Type I from bovine brain)], L-α-phosphatidylinositol (PI), L-α-phosphatidylinositol-4-phosphate (PIP), L-α-phosphatidylinositol-4,5-biphosphate (PIP_2), and L-α-lysophosphatidylcholine (from egg yolk) were purchased from Sigma Chemical Company (St. Louis, MO). All the phospholipids were tested for purity by thin-layer chromatography. Mixed

phosphoinositides, N-2-hydroxymethylpiperazine-N-2'-ethane sulfonic acid (HEPES), sucrose, Ficoll, and dithioerythritol (DTE) were also obtained from Sigma. All other chemicals and reagents were of the highest analytical grade.

2.2 Preparation of Synaptic Membranes.

Synaptosomal membranes were prepared by modifying[14] the method of Cotman and Matthews.[15] Whole brains minus cerebellum and brain stem were quickly removed following decapitation of male Sprague-Dawley rats weighing 150-200 g. A crude P_2 pellet obtained by standard differential centrifugation technique[15] was resuspended in 0.32 M of sucrose and layered over a discontinuous gradient composed of 7.5 and 12% Ficoll in 0.32 M of sucrose. Following centrifugation at 65,000 g for 60 min, synaptosomes were collected from the 7.5 to 12% interphase and lysed in 20 mM of Tris, pH 8.5, containing 0.5 mM of DTE for 60 min at 4 °C. The lysed synaptosomal membranes were pelleted by centrifugation at 37,000 g and resuspended in lysing buffer (for ATPase assay) or in other buffers (for phospholipases treatment) to a protein concentration of 1 mg/mL. Protein was determined by the method of Lowry et al.[16]

2.3 Assay of ATPase Activity.

ATPase activity was measured as described previously[4] at 37 °C in a 2-mL final volume containing 20 mM of HEPES, 100 mM of KCl, 250 µM of $MgCl_2$, 100 µM of ethylenebis(oxyethylene-nitrilo)tetraacetic acid (EGTA), 95 µM of $CaCl_2$ (2.49 µM of free Ca^{++}),[17] 200 µM of ATP and 100 µM of synaptosomal protein at pH 7.4. Intrinsic ($Na^+ + K^+$)-ATPase and contaminating mitochondrial Mg^{++}-ATPase were controlled by including 1 mM of ouabain and 10 mM of sodium azide, respectively.[1] The reaction was initiated with the addition of ATP and was quenched after 3 min with 200 µL of 6N HCl. Aliquots were taken for Pi determination by the method of Lanzetta et al.[18] Suitable blanks are run in the absence of Mg^{++} or Ca^{++}. Mg^{++}-ATPase activity was expressed as the difference between the incubations in the presence and absence of Mg^{++}. Ca^{++}/Mg^{++}-ATPase activity was determined by the difference in the activity in the presence of Ca^{++} and Mg^{++} minus activity in the presence of Mg^{++}.

2.4 Phospholipase Treatment.

For phospholipases A_2, C, and D treatments, the synaptosomal membranes were suspended in 0.32 M of sucrose - 10 mM of HEPES, pH 7.4, containing 0.87% of NaCl and 10 mM of CaCl; 0.32 M of sucrose - 10 mM of HEPES, pH 7.4, and in 0.32 M of sucrose - 10 mM of HEPES, pH 6.0, containing 0.87% of NaCl - 10 mM of CaCl, respectively. For treatment with phospholipase A_2 in the presence of albumin, the synaptosomal membranes were suspended in 0.32 M of sucrose - 10 mM of HEPES, pH 7.4, containing 0.87% of NaCl, 10 mM of CaCl, and 1% of bovine albumin (essentially fatty acid and globulin free from Sigma).

Phospholipases were added, and tubes were incubated at 35 °C for 15 min in a shaking water bath. Tubes were then quickly cooled on ice, centrifuged, and the pellet washed three times with 0.32 M of sucrose - 10 mM of HEPES, pH 7.4. Finally, the pellet was suspended in 20 mM of Tris - 0.5 mM of DTE, pH 8.5, at a protein concentration of 1 mg/mL. All buffers contained 50 µg/mL of phenylmethylsulfonylfluoride.

2.5 Extraction and Analysis of Phospholipids.

Phospholipids were extracted from synaptosomal membranes by the method of Martin et al.[19] To 1 mL of synaptosomal suspension, 1.4 mL of methanol and 1.4 mL of 2-butanol were added, followed by 2.8 mL of chloroform and 1.4 mL of water. Tubes were mixed thoroughly, and the phases were separated in a clinical centrifuge. The lower phase was aspirated, and the upper phase was extracted again with 1 mL of chloroform. Combined lower phases were dried under nitrogen and dissolved in 1 mL of chloroform-methanol [1:1 volume by volume ratio (v/v)]. A phosphorus assay was carried out as described by Bartlett.[20]

2.6 Phospholipid Dispersion.

Phospholipids dissolved in chloroform-methanol (1:1 v/v) or in chloroform were taken, and the organic solvents were evaporated under nitrogen. They were then dispersed in the assay buffer by sonication in a heat system sonicator, Model W-220 [Ultrasonic, Incorporated (Jericho, NY)] equipped with a microtip at 0 °C under nitrogen. They were added to the incubations at the indicated concentrations. Preincubations with the synaptosomal membranes were carried out for 10 min before adding ATP.

3. RESULTS AND DISCUSSION

3.1 Effect of Phospholipases.

The effect of phospholipases A_2, C, and D treatments on rat brain synaptosomal Ca^{++}/Mg^{++}-ATPase and Mg^{++}-ATPase was studied. Table 1 shows the results of such treatment. Longer incubations with phospholipase A_2 had inhibitory effects on the enzyme activities, presumably because of extensive hydrolysis of phospholipids and increased concentration of lysophospholipids.

An increase in the activities of Ca^{++}-ATPase (35%), as well as Mg^{++}-ATPase (15%), was observed when treating the synaptosomal membrane with phospholipase A_2 (Table 1) with 45, 29, and 39% hydrolysis of L-α-phosphatidylcholine (PC), L-α-phosphatidylserine (PS), and PE, respectively. Sphingomyelin concentration was unaffected. When fat-free albumin was included during phospholipase A_2 treatment of synaptosomal membranes and followed by washing with buffer containing albumin, no change in the enzyme activities (Table 1) occurred.

Table 1. Effect of Phospholipases A, C, and D on Mg^{++}-ATPase and Ca^{++}/Mg^{++}-ATPase.*

Treatment	Control	Treated	p Value
Mg^{++}-ATPase			
Phospholipase A	131 ± 20	151 ± 19	<0.05
Phospholipase A	136 ± 12	132 ± 11	N.S.**
(In presence of albumin)			
Phospholipase C	143 ± 19	90 ± 20	<0.001
Phospholipase D	140 ± 21	109 ± 16	<0.05
Ca^{++}/Mg^{++}-ATPase			
Phospholipase A	59 ± 15	80 ± 19	<0.01
Phospholipase A	60 ± 10	58 ± 8	N.S.
(In presence of albumin)			
Phospholipase C	63 ± 11	42 ± 8	<0.001
Phospholipase D	58 ± 10	67 ± 11	N.S.

*Synaptosomal membranes were treated with phospholipases, and the enzyme activities were measured as described in METHODS. Values expressed as moles Pi liberated per milligram/protein/minute are the means of 10 determinations S.D. The p value was determined from student's t-test. For treatment with phospholipase A_2 in the presence of albumin, there were five determinations, each done in triplicate.

**Not significant.

Treating synaptosomal membranes with phospholipase C caused a significant decrease in the activities of Ca^{++}/Mg^{++}-ATPase (33%) and Mg^{++}-ATPase (37%) (Table 1). The effect of phospholipase C on various phospholipids is shown in Table 2. Hydrolysis of PC, PE, and PS to 60, 32, and 43%, respectively, was seen with a 27% increase in PA and no effect on sphingomyelin and PI. It has been reported before that PI does not react with phospholipase C from Bacillus cereus.[21,22] Figure 1 shows the effect of phospholipase C concentration on phospholipid hydrolysis and ATPases. At low concentrations of 0.25 U of phospholipase C per milligram of protein, a 40% loss of phospholipids occurred with nearly the same percentage loss in the activities of Ca^{++}/Mg^{++}-ATPase and Mg^{++}-ATPase. An increase in the phospholipase C concentration, up to 1 unit/mg of synaptosomal protein, did not lead to any further significant

Table 2. Effect of Phospholipase C on Individual Phospholipids of Rat Brain Synaptosomes (Values Expressed as Percentage of Control).*

Phospholipid	Control	Phospholipase C-Treated
Total	100	49
PC	100	38
PE	100	68
Sphingomyelin	100	100
PI	100	98
PS	100	57
PA	100	129

*Phospholipids extracted, as described in METHODS, were separated by two dimensional, thin-layer chromatography (TLC) on silica gel H plates [Analtech, Incorporated (Newark, DE)] using chloroform-methanol-20% methylamine (60:36:10 V/V) as solvent systems. For total phospholipid, an aliquot was applied to a corner not wet by solvent. Spots were visualized by exposure to iodine vapors, outlined, scraped, and phosphorus was assayed by Bartlett's method.[20] Unless otherwise indicated, all the values are the averages of at least three separate experiments each done in triplicate.

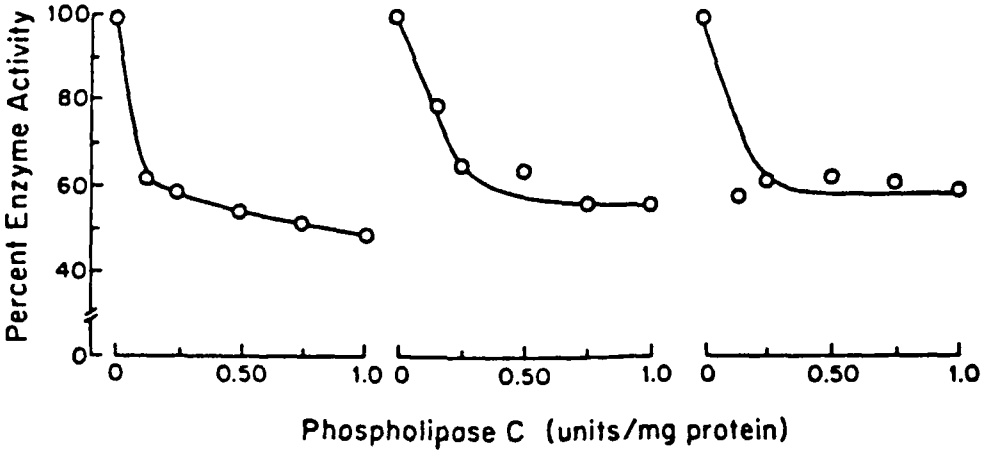

Figure 1. Effect of Phospholipase C on Phospholipid, Ca^{++}/Mg^{++}-ATPase and Mg^{++}-ATPase of Rat Brain Synaptosomal Membranes.*

*Synaptosomal membranes were incubated with phospholipase C at indicated concentrations, washed, and used for the enzyme assay after suspension in 20 mM of Tris-0.5 mM of DTE, pH 8.5, as described in METHODS. Left, middle, and right panels show the effect of phospholipase C on total phospholipids, Ca^{++}/Mg^{++}-ATPase and Mg^{++}-ATPase, respectively. Each value is the average of five experiments.

change either in phospholipid hydrolysis or enzyme activities. All the experiments on reactivation studies with phospholipids were carried out after treatment of synaptosomal membranes that affected the phospholipids and the enzyme activities (Figure 1 and Tables 1 and 2).

When synaptosomal membranes were treated with phospholipase D, there was a significant decrease in the Mg^{++}-ATPase activity (Table 1) with a 150% increase in the phosphatidic acid content (data not shown).

3.2 Effect of Phospholipids.

All the phospholipids used in these studies were tested for purity by TLC on silica gel H plates using chloroform-methanol-ammonia (65:35:5) and ran as single spots.

Lysophospholipids are the by-products of phospholipase A_2 treatment. As shown in Figure 2, lysophosphatidylcholine inhibited Ca^{++}/Mg^{++}-ATPase and Mg^{++}-ATPase activities of synaptosomal membrane. The extent of inhibition at high concentrations of lysophosphatidylcholine was higher for Ca^{++}/Mg^{++}-ATPase than for Mg^{++}-ATPase.

Experiments on reactivation of Ca^{++}/Mg^{++}-ATPase and Mg^{++}-ATPase by added lipids after phospholipase C treatment were carried out to see which of the phospholipids were able to reverse the inhibition of the enzyme activities. Figure 3 shows the effect of PC, PE, and PS on the Ca^{++}/Mg^{++}-ATPase (right panel) and Mg^{++}-ATPase (left panel) activities of phospholipase C treated synaptosomal membranes. L-α-phosphatidylethanolamine did not seem to have any effect on the enzyme activities. Only 5% stimulation of Mg^{++}-ATPase activity of the untreated membranes and 10% activation of the phospholipase C treated membranes were observed with PC. Twenty-five percent stimulation and reactivation of Ca^{++}/Mg^{++}-ATPase activity was obtained by PS.

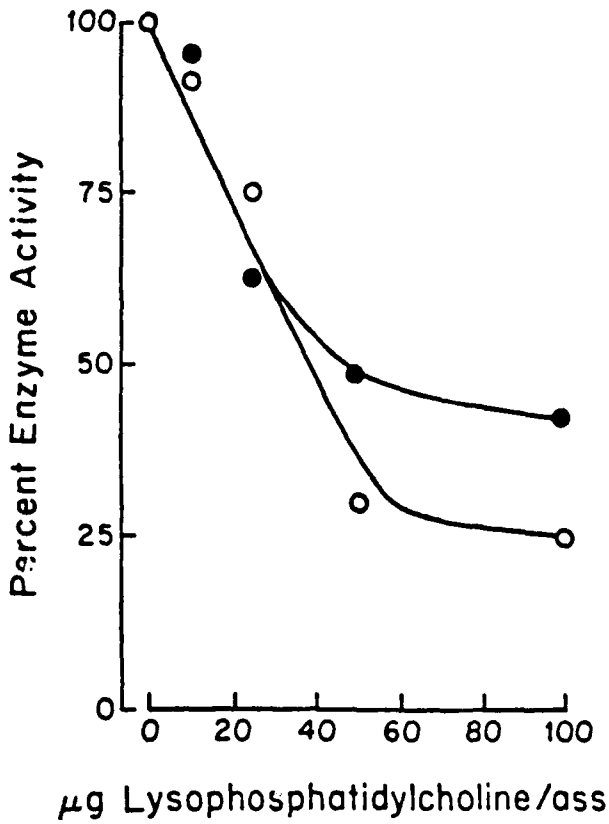

Figure 2. Effect of Lysophosphatidylcholine on Ca^{++}/Mg^{++}-ATPase and Mg^{++}-ATPase of Rat Brain Synaptosomal Membranes.*

*Lysophosphatidylcholine sonicated in the assay buffer was added to the incubations at indicated concentrations. Preincubation with phospholipid was carried out for 10 min before adding ATP. Enzyme activity is expressed as percentage of the control value. Unless otherwise indicated, values presented here and all other experiments are averages of at least 3 separate experiments, and the variation was within 10%.

0——0 Ca^{++}/Mg^{++}-ATPase

●——● Mg^{++}-ATPase

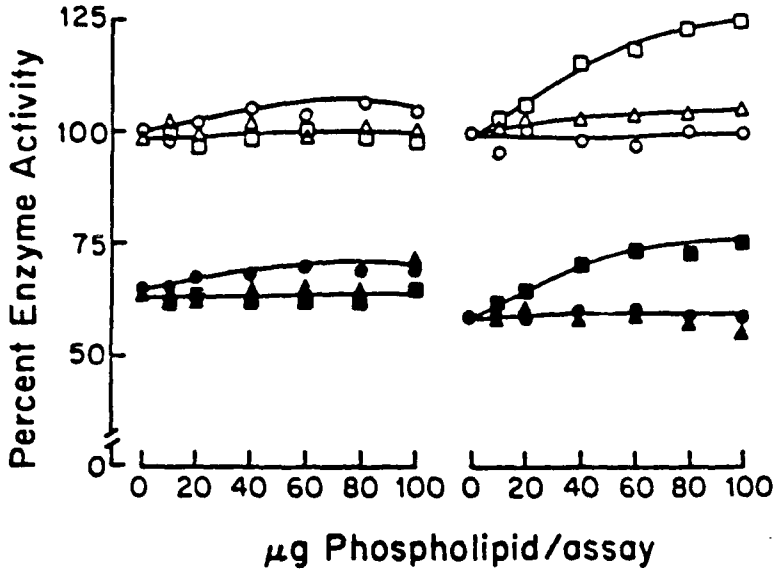

Figure 3. Effect of Phosphatidylcholine, Phosphatidylethanolamine, and Phosphatidylserine on Phospholipase C Treated Synaptosomal Ca^{++}/Mg^{++}-ATPase (right panel) and Mg^{++}-ATPase (left panel).*

*Treatment with phospholipase C and assay conditions were exactly as described in METHODS. Sonicated phospholipids in assay buffer were added to the incubations containing control and phospholipase C treated synaptosomal membranes at indicated concentrations. Preincubation with phospholipids was carried out for 10 min before adding ATP. Open symbols represent control enzyme activities, whereas closed symbols represent activities in phospholipase C treated membranes.

○——○ ●——● PC

△——△ ▲——▲ PE

□——□ ■——■ PS

Diacylglycerol (DAG), produced as a result of phospholipase C action on phospholipids, is rapidly phosphorylated to PA.[23,24] Diacylglycerol did not have any effect on either Mg^{++}-ATPase or Ca^{++}/Mg^{++}-ATPase on control or phospholipase C treated membranes (data not shown). On the other hand, PA strongly inhibited Mg^{++}-ATPase activity. Compared to phospholipase C treated membranes (40%), the extent of such inhibition was very high in untreated membranes (55%). L-α-Phosphatidic acid stimulated the Ca^{++}/Mg^{++}-ATPase activity by 12% in untreated and reactivated it from 40 to 57% of control value in the phospholipase C treated membranes.

Phosphoinositides (mixture of PI, PIP, and PIP_2) inhibited Mg^{++}-ATPase activity in untreated (25% inhibition) and phospholipase C treated (22% inhibition) synaptosomal membranes (Figure 4, left panel). Some stimulation of the Ca^{++}/Mg^{++}-ATPase activity (22%) of the untreated membranes by phosphoinositides was observed, but the two enzyme activities were inhibited further in phospholipase C treated membranes (Figure 4, right panel). When synaptosomal membranes were treated with phospholipase A_2 in the presence of albumin, no change in the enzyme activities (Table 1) occurred. These data indicate that after partial lipid hydrolysis, the remaining phospholipids could maintain the enzyme activities and that the phospholipid core around the enzyme is required for the enzymes to be active. Stimulation of the enzyme activities upon treatment with phospholipase A in the absence of albumin may be caused because of liberated fatty acids and lysophospholipids. Stimulation of Ca^{++}/Mg^{++}-ATPase activity with long chain unsaturated fatty acids has been reported.[25-27] Niggli et al.[25] and Hanahan and Nelson[28] suggest that one of the necessary requirements for stimulation of Ca^{++}/Mg^{++}-ATPase is a negative charge around the enzyme based on the observation that Ca^{++}/Mg^{++}-ATPase reconstituted in phosphotidylcholine vesicles is stimulated by calmodulin, but purified ATPase in phosphotidylserine vesicles is not because the enzyme is already maximally active. Stimulation of the enzyme activity observed in the present investigation may be partly due to an increased negative charge. Another possibility could be the permeability changes in the membrane caused by such treatment. Lysophosphatidylcholine inhibits the enzyme activities (Figure 2). Because lyso PC is one of the hydrolytic products of phospholipase A_2 treatment, one would expect that such treatment should inhibit the enzyme activities. However, lyso PC is not the only lysophospholipid liberated. According to a recent report by Tokumura et al.,[29] lysophospholipids, depending on the chain length, degree of unsaturation as well as their nature, can stimulate or inhibit the enzyme activity. From the above and from similar results obtained by Mostafa et al.[9] with human erythrocyte and rabbit platelet Ca^{++}/Mg^{++}-ATPase, it appears that a critical concentration of hydrolytic products of phospholipase A treatment, and also the extent of hydrolysis of phospholipids, govern the Ca^{++}/Mg^{++}-ATPase activity of the synaptosomal membranes.

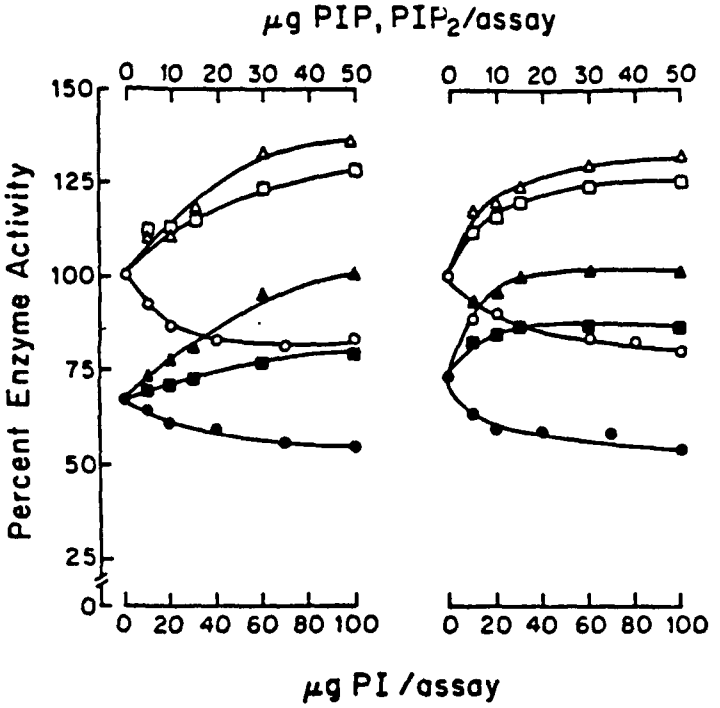

Figure 4. Effect of Individual Phosphoinositides on Phospholipase C Treated Rat Brain Synaptosomal Ca^{++}/Mg^{++}-ATPase (Right Panel) and Mg^{++}-ATPase (Left Panel).*

*Experimental procedure and assay conditions were exactly as described in Figure 3. Enzyme activities are expressed as the percentage of control values. Open symbols represent control enzyme activities whereas closed symbols represent enzyme activities in phospholipase C treated synaptosomes.

○——○ ●——● PI

△——△ ▲——▲ PIP

□——□ ■——■ PIP_2

3.3 Effect of Phospholipase C.

Treating synaptosomal membranes with phospholipase C inhibited Ca^{++}/Mg^{++}-ATPase as well as Mg^{++}-ATPase activities (Figure 1). We found that phospholipase C from Bacillus cereus hydrolyzed PC, PE, and PS with no effect on PI and sphingomyelin (Table 2). When these lipids were added back, PS and PC stimulated Ca^{++}/Mg^{++}-ATPase and Mg^{++}-ATPase activities, respectively (Figure 3). Recently, Philipson et al.[30] reported that phospholipase C from Clostridium perfringeus preferentially hydrolyzed PC, PE, and sphingomyelin leaving negatively charged phospholipids (PI and PS) intact. This treatment resulted in stimulation of Na^{+}-dependent Ca^{++} uptake when 1070% of the purified canine cardiac sarcolemmal membrane phospholipid was hydrolyzed[30] indicating the dependence of such an exchange on

negatively charged phospholipids. From these results and the results obtained by us and others,[9,10,31] it appears that the maintenance of Ca^{++} transport is strongly dependent on acidic phospholipids. Although inhibition of $(Ca^{++} + Mg^{++})$-ATPase activity with phospholipase C and its subsequent reactivation with lysolecithin has been reported,[6] Ronner et al.,[7] failed to get such reactivation of $(Ca^{++} + Mg^{++})$-ATPase of erythrocytes treated with phospholipase A. They suggested that reactivation obtained by Coleman and Bramley[6] could be due to rearrangement (caused by lysolecithin) of the remaining lipids in the membranes.

Exogeneously added PA inhibited Mg^{++}-ATPase activity. Roelofsen and van Deenen[32] obtained similar results with erythrocyte Mg^{++}-ATPase. They also observed inactivation of Mg^{++}-ATPase of erythrocyte on complete hydrolysis of PC, PE, and PS with phospholipase C. Inactivating Mg^{++}-ATPase with phospholipase D treatment (Table 1) strongly supports the inhibitory effect of PA on this enzyme and provides a possible explanation for such inactivation after phospholipase C treatment because PA is rapidly formed as a result of phosphorylation of DAG.[23,24]

3.4 Effect of Acidic Phospholipids.

Acidic phospholipids play a regulatory role in the membrane Ca^{++}/Mg^{++}-ATPase. In these experiments, we observed reactivation of Ca^{++}/Mg^{++}-ATPase from partially delipidated synaptosomal membranes with PIP_2, PIP, PA, and PS with PIP_2 being most effective (Figures 4 and 5). On the other hand, mixed phosphoinositides stimulated Ca^{++}/Mg^{++}-ATPase of the untreated synaptosomal membranes but inhibited the Mg^{++}-ATPase activity of untreated and phospholipase C treated membranes and Ca^{++}/Mg^{++}-ATPase of treated membranes (Figure 5). Activation of erythrocyte $(Ca^{++} + Mg^{++})$-ATPase with mixed phosphoinositides has been reported by Mostafa et al.[9] When individual phosphoinositides were added to the incubations, PI surprisingly inhibited Ca^{++}/Mg^{++}-ATPase and Mg^{++}-ATPase activities. Choquette et al.[10] observed inhibition of purified $(Ca^{++} + Mg^{++})$-ATPase with PI in the presence of calmodulin. Nelson and Hanahan[31] reported several-fold activation of this enzyme with the mixture of PS and PI with Triton N101. Although, from above, it is tempting to suggest the presence of PI in the mixed phosphoinositides being inhibitory for the enzyme activities (Figure 5), stimulation of Ca^{++}/Mg^{++}-ATPase of the untreated synaptosomal membranes (Figure 5) cannot be explained.

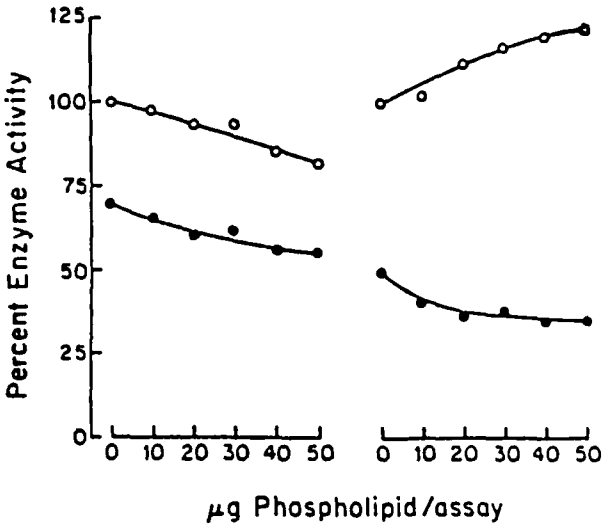

Figure 5. Effect of Mixed Phosphoinositides on Phospholipase C Treated Synaptosomal Ca^{++}/Mg^{++}-ATPase (Right Panel) and Mg^{++}-ATPase (Left Panel).*

*Experimental conditions and assay procedures were exactly as described under the legend for Figure 3. Enzyme activities are expressed as the percentage of control values. Open circles represent control enzyme activities; whereas, closed circles represent enzyme activities in phospholipase C treated membranes.

Complete reactivation of the enzyme activities could not be obtained with externally added phospholipids, except for PIP. PIP_2 and PIP occur in trace amounts in most tissues, but in nerve tissues, their concentrations are higher and, together, these phosphoinositides account for as much lipid phosphorus as PI.[33,34] It is also known that there is a rapid hydrolysis of L-α-phosphatidylinositol-4,5-biphosphate and PIP postmortem. Substantial stimulation of the enzyme activities in the untreated membranes with PIP_2 and PIP and their reactivation in phospholipase C treated membranes may be explained in light of these observations.

4. CONCLUSIONS

Treatment with phospholipase A_2 caused an increase in the activities of Ca^{++}/Mg^{++}-ATPase and Mg^{++}-ATPase; whereas, with phospholipase C treatment, both the enzyme activities were inhibited. Phospholipase D treatment had no effect on Ca^{++}/Mg^{++}-ATPase, but Mg^{++}-ATPase activity was inhibited. Inhibition of Mg^{++}-ATPase activity after phospholipase C treatment was relieved by adding PIP_2 and to a lesser extent with PIP and PC. Phosphatidylserine, PA, PIP, and PIP_2 brought about the reactivation of Ca^{++}/Mg^{++}-ATPase. Phosphatidylinositol and PA inhibited Mg^{++}-ATPase activity. The Michaelis constants (k_{ms}) for Ca^{++} (0.47 μM) and Mg^{++} (60 μM) of the enzyme were unaffected after treatment with the phospholipases.

LITERATURE CITED

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